

“Broken along the line 2nd rate Land generally, Lime stone rock along the ravines”.

The largest concentration of *A. ruta-muraria* at this site occurs in a small, exposed area at the eastern terminus of a south-facing outcrop. On the south and east facing bluffs, *A. ruta-muraria* grows with the dominant fern encountered on these outcrops, *Pellaea glabella* Mett. ex Kuhn (smooth cliff-brake), whereas on the small, sheltered northern face it occurs with *Cystopteris bulbifera* (L.) Bernh. (bulblet fern). Other plant species co-inhabiting the shelves and crevices of these bluffs include *Arabis lyrata* (rock cress), *Euphorbia maculata* L. (spurge), *Minuartia michauxii* (Fern.) Farw. (= *Arenaria stricta* Michx.; rock sandwort), *Nepeta cataria* L. (catnip), *Parietaria pensylvanica* Muhl. ex Willd. (pellitory), *Aquilegia canadensis* L. (columbine), *Sporobolus neglectus* Nash (small rush grass) and *Schizachyrium scoparium* (little bluestem).

We thank Gart Bishop, Doug Larson, Jim Peck, Tony Reznicek, Carl Taylor, and George Yatskievych for information or advice.—THOMAS F. CADY, Iowa City, IA and DIANA HORTON, Biological Sciences Department, 143 BB, University of Iowa, Iowa City, IA 52242.

Vitexin 7-*O*-rhamnoside, a New Flavonoid from *Pteris vittata*.—Previous work on the flavonoids of *Pteris vittata* L. has led to the identification of luteolinidin 5-*O*-glucoside by Harborne (Phytochemistry 5:589–600, 1966); in addition acid hydrolysis of extracts of this fern has led to the identification of kaempferol, quercetin, leucocyanidin and leucodelphinidin by Voirin (Ph. D. thesis, University of Lyon, p. 151, 1970). More recently 3-*C*-(6'''-acetyl- β -cellobiosyl)-apigenin (Amer. Fern J. 89:217–220, 1999) and 6-*C*- β -cellobiosyl-isoscutellarein-8-methyl ether together with quercetin 3-*O*-glucuronide and rutin (Amer. Fern J. 90:42–45, 2000) have been identified by Imperato and Telesca. In addition three kaempferol glycosides (3-*O*-glucoside, 3-*O*-glucuronide and 3-*O*-(X'',X''-di-protocatechuoyl)-glucuronide), two di-*C*-glycosylflavones (3,8-di-*C*-arabinosylluteolin and 6-*C*-arabinosyl-8-*C*-glucosylluteolin) and three flavonol glucosides acylated with hydroxycinnamic acids (kaempferol and quercetin 3-*O*-(2'', 3''-di-*O*-*p*-coumaroyl)-glucosides together with kaempferol 3-*O*-(X''-*O*-*p*-coumaroyl-X''-*O*-feruloyl)-glucoside) have been found by Imperato (Amer. Fern J. 90:141–144, 2000; Amer. Fern J. 92:244–246, 2002; Amer. Fern J. 93:157–160, 2004).

In the present paper two flavonoids (I and II) have been isolated from aerial parts of *Pteris vittata* L. collected in the Botanic Garden of the University of Naples. The fern was identified by Dr. R. Nazzaro (University of Naples); a voucher specimen (149.001.001.01) has been deposited in the Herbarium Neapolitanum (NAP) of the University of Naples.

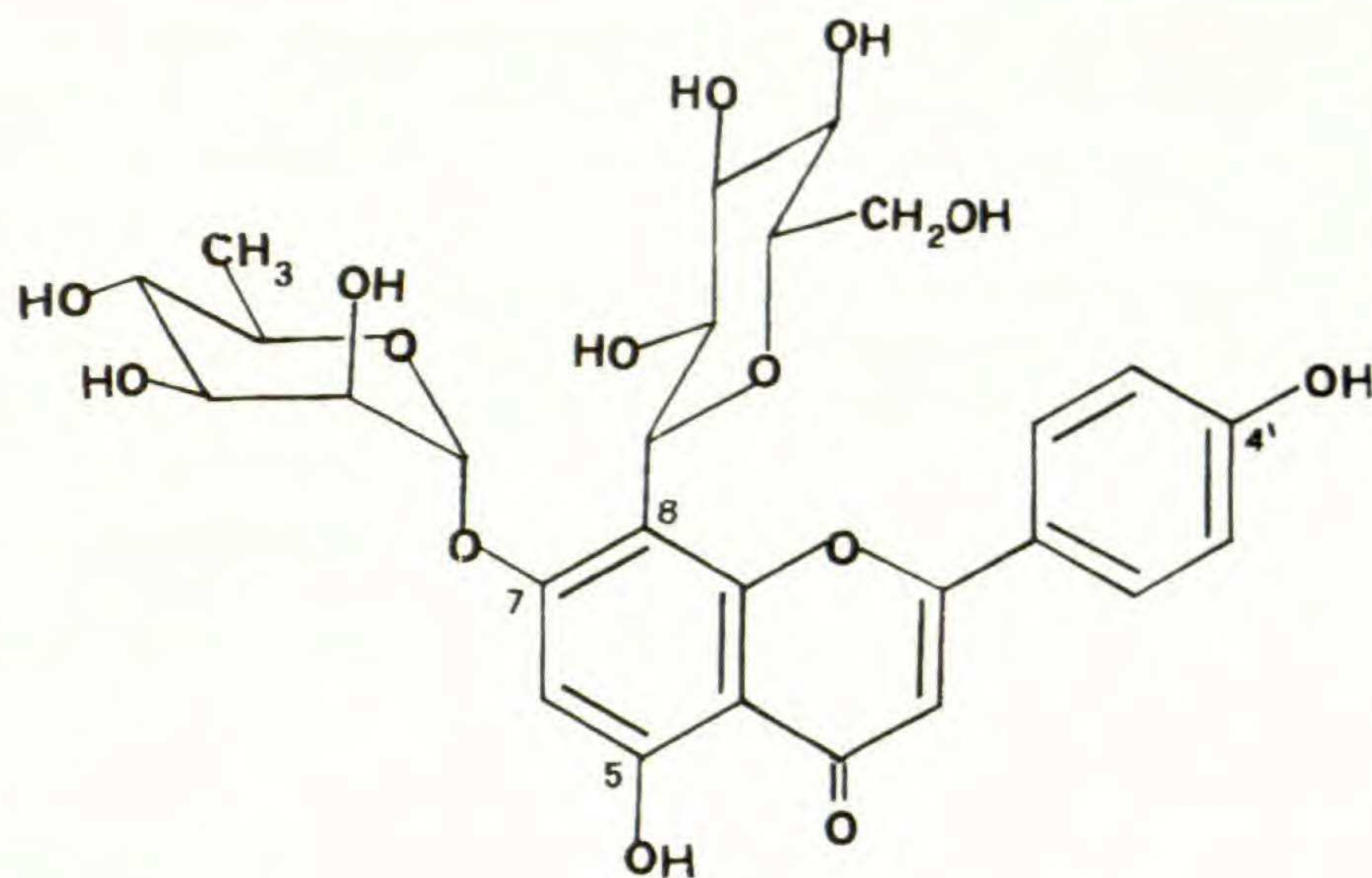
Flavonoids (I and II) were isolated from an ethanolic extract of aerial parts of *Pteris vittata* by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid) and BEW (*n*-butanol-

TABLE 1. ^{13}C - and ^1H -NMR spectral data (DMSO- d_6) of flavonoid (I).

Carbon	δ_c ppm	δ_H ppm (J in Hz)
Apigenin		
2	164.6	0.97 (3H, d, J = 6, rhamnosyl methyl group)
3	102.7	2.95–4.11 (10 H, m, glucosyl 6 protons + rhamnosyl 4 protons)
4	182.4	4.52 (1 H, d, J = 8, glucosyl H-1)
5	161.6	5.51 (1 H, d, J = 2, rhamnosyl H-1)
6	99.1	6.33 (1 H, s, H-6)
7	163.2	6.77 (1 H, s, H-3)
8	104.4	6.91 (2 H, d, J = 8.6, (??) H-3' and H-5')
9	156.8	7.97 (2 H, d, J = 8.6, H-2' and H-6')
10	103.2	
1'	121.6	
2',6'	128.9	
3',5'	115.7	
4'	160.5	
<i>O</i> -Rhamnosyl		
1''	99.2	
2''	70.4 ^a	
3''	70.5 ^a	
4''	71.8 ^b	
5''	70.8 ^b	
6''	18.2	
<i>C</i> -Glucosyl		
1'''	73.6	
2'''	71.5 ^b	
3'''	79.6	
4'''	69.9	
5'''	82.1	
6'''	62.2	

^{a,b} Assignments with the same superscripts may be interchanged.

ethanol-water, 4:1:2.2). Further purification was carried out by Sephadex LH-20 column chromatography eluting with methanol. Color reactions (brown to yellow in UV+NH₃), chromatographic behaviour (*R_f* values on Whatman No 1 paper: 0.37 in BAW; 0.54 in 15% HOAc) and ultraviolet spectral analysis in the presence of usual shift reagents (λ_{max} (nm) (MeOH) 273, 332; +AlCl₃ 280, 302, 344, 383; +AlCl₃/HCl 281, 300, 341, 381; +NaOAc 273, 391; +NaOMe 274, 391) suggested that flavonoid (I) may be a flavonoid glycoside with free hydroxyl groups at positions 5 and 4'. Acid hydrolysis (2N HCl; 1 hr at 100°C) gave L-rhamnose, vitexin (8-*C*-glucosylapigenin) and isovitexin (6-*C*-glucosylapigenin). These results show that flavonoid (I) may be a *C*-glucosylapigenin 7-*O*-rhamnoside and this was confirmed by the FAB mass spectrum which showed a quasimolecular ion at *m/z* 579 [M+H]⁺ and an ion at *m/z* 601 [(M+H)+Na]⁺. ^1H - and ^{13}C - NMR spectral data (Table 1) showed that D-glucose is attached at position 8 of the flavone since H-6 appeared as a singlet at δ 6.33; in addition C-8 showed a downfield shift of 10.4 p.p.m., in comparison with the corresponding carbon atom of apigenin; this shift of glycosylated carbon

FIG. 1. Vitexin 7-*O*-rhamnoside.

atom is an effect of *C*-glycosylation on the aglycone spectrum as shown in the review by Markham and Chari (pp. 19–134 in J. B. Harborne and T. J. Mabry, eds., *The Flavonoids: Advances in Research*, Chapman and Hall, London, 1982). Hence the presence of isovitexin among the products of acid hydrolysis of flavonoid (I) is due to a Wessely-Moser acid isomerization. The combined data show that flavonoid (I) is vitexin 7-*O*-rhamnoside (Fig 1), a new natural product; ^1H - and ^{13}C -NMR spectral data (Table 1) support this structure.

A large number of *C*-glycosylflavonoids have been found in ferns as shown in the review by Markham (pp. 427–468 in J. B. Harborne, ed., *The Flavonoids, Advances in Research Since 1980*, Chapman and Hall, London, 1988) and in a review by Imperato (pp. 39–75 in *Current Topics in Phytochemistry*, Research Trends, Trivandrum, 2000). However *C*-glycosylflavonoid *O*-glycosides are rare in ferns. A *C*-glycosylflavonoid *O*-glycoside in which the hydrolyzable sugar is attached to a phenolic hydroxy group was found for the first time in ferns by Hiraoka (*Bioch. Syst. and Ecol.* 6:171–175, 1978) who identified vitexin 7-*O*-glucoside in the genus *Dryopteris*; subsequently 8-*C*-rhamnosylluteolin 7-*O*-rhamnoside was found in *Pteris cretica* (*Phytochemistry* 37:589–590, 1994) by Imperato. *C*-Glycosylflavonoid-*O*-glycosides in which the hydrolysable sugar is attached to a hydroxy group of a *C*-glycosyl moiety were found for the first time in ferns by Markham and Wallace (*Phytochemistry* 19:415–420, 1980) who found apigenin and luteolin 8-*C*-glucoside 2''-*O*-xylosides in *Trichomanes venosum*; two further *C*-glycosylflavone *O*-glycosides of this type have subsequently been found in *Pteris vittata* by Imperato and Telesca who identified these flavonoids as 3-*C*-(6'''-acetyl-cellobiosyl)-apigenin (*Amer. Fern J.* 89:217–220, 1999) and 6-*C*- β -cellobiosyl-isoscutellarein 8-methyl ether (*Amer. Fern J.* 90:42–45, 2000).

Flavonoid (II) has been identified as kaempferol 3-*O*-rutinoside by UV spectral analysis with the customary shift reagents, acid hydrolysis, ^1H -NMR

spectrum, ^{13}C -NMR spectrum and co-chromatography with an authentic sample. As shown in the review by Markham (1988), kaempferol 3-*O*-rutinoside has previously been identified in *Adiantum capillus-veneris* (Adiantaceae), another of the 53 species of *Adiantum*, *Loxsonia cunninghamii*, *L. costaricensis* (Loxsoniaceae), all four species of *Bommeria* (Sinopteridaceae), four species of *Gymnopteris* (Sinopteridaceae), two species of *Hemionitis* (Sinopteridaceae) and the genus *Trachypteris* (Sinopteridaceae); more recently kaempferol 3-*O*-rutinoside has been identified in *Diplazium nipponicum* (Athyriaceae) and *Thelypteris palustris* (Sinopteridaceae) as shown in the review by Imperato (2000).

The author thanks Murst (Rome) for financial support. Mass spectral data were provided by SESMA (CNR, Naples).—FILIPPO IMPERATO Dipartimento di Chimica, Università della Basilicata, 85100 Potenza, Italy.